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(57) Process for the preparation of N-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester.

(57) To produce N-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester there is carried out a reaction of N-formyl-L-aspartic acid anhydride with L-phenylalanine methyl ester in a reaction solvent and in the presence of acetic acid and formic acid and in a complete mixing type continuous reaction mode. By this process, the amount of the desired ester produced, relative to its  $\beta$ -isomer, is increased.

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Process for the production of N-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester

This invention relates to a process for obtaining N-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester (hereinafter referred to as PM) and N-formyl-L-aspartic acid anhydride.

$\alpha$ -L-Aspartyl-L-phenylalanine methyl ester - (hereinafter referred to as  $\alpha$ -APM) is known as an excellent sweetening agent. As a process for its production, there is known a process which comprises subjecting N-formyl-L-aspartic acid anhydride and PM to a condensation reaction in an organic solvent to form N-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester (hereinafter referred to as For- $\alpha$ -APM) and then removing the formyl group from this reaction product to obtain the desired  $\alpha$ -APM (GB-A-2153365).

However in practice, the desired For- $\alpha$ -APM is not the sole product since its isomer, N-formyl- $\beta$ -L-aspartyl-L-phenylalanine methyl ester (hereinafter referred to as For- $\beta$ -APM), is also produced, and the ratio of For- $\alpha$ -APM to For- $\beta$ -APM (hereinafter referred to the  $\alpha$ : $\beta$  ratio) is not always satisfactory, because, even when the practical concentrations of reactants as given in GB-A-2153365 (0.2 to 0.8 M/litre) are employed, the  $\alpha$ : $\beta$  ratio is small (i.e. 1.8:1 to 3.3:1), and therefore the process cannot be regarded as an efficient production process. Indeed, when low concentrations of the reactants according to GB-A-2153365 (0.025 M/litre) are employed, a considerably high  $\alpha$ : $\beta$  ratio of 5.5 is attained. In this case, large reaction vessels are needed. However, such large reactant vessels are not practical from the industrial point of view. On the contrary, according to the present invention, the  $\alpha$ : $\beta$  ratios can be remarkably enhanced at the practical concentrations of the reactants.

The production of For- $\beta$ -APM as a by-product reduces not only the yield of For- $\alpha$ -APM but also that of the end product,  $\alpha$ -APM. This also means that the  $\alpha$ -APM is contaminated with  $\beta$ -L-aspartyl-L-phenylalanine methyl ester (hereinafter referred to as  $\beta$ -APM). Since  $\beta$ -APM does not exhibit sweetness,  $\alpha$ -APM contaminated with  $\beta$ -APM must be purified to remove the  $\beta$ -APM. Therefore, as described above, an increase in the  $\alpha$ : $\beta$  ratio is believed to be very advantageous in order that the desired end product,  $\alpha$ -APM, may be obtained in a good yield.

The present inventors have intensively studied in order to overcome this disadvantage and, as a result, have surprisingly discovered that by reacting N-formyl-L-aspartic acid anhydride and PM in a reaction medium in the presence of acetic acid or

of a mixture of acetic acid and formic acid in a "complete mixing type continuous reaction mode", the  $\alpha$ : $\beta$  ratio is greatly enhanced, and therefore have accomplished this invention.

The expression "complete mixing type continuous mode" used herein means that the starting materials (e.g. N-formyl-L-aspartic acid anhydride and PM) are continuously fed to a reaction zone - (e.g. a reactor) whilst the mass in the reaction zone (e.g. the mass in the reactor) is being completely mixed (e.g. by stirring) and whilst the resulting reaction mixture is being continuously withdrawn from the reaction zone (e.g. by being allowed to overflow from the reactor).

The N-formyl-L-aspartic acid anhydride used in the process of this invention may be obtained by reacting formic acid and acetic anhydride with L-aspartic acid.

The reaction solvent used in the process of this invention may be any solvent as long as it is not particularly reactive with the reactants and the reaction product. Representative examples thereof include hydrocarbons such as toluene, xylene, hexane, etc.; esters such as ethyl acetate, methyl propionate, etc.; carboxylic acids such as acetic acid, propionic acid, etc.; ketones such as acetone, methyl ethyl ketone, etc.; halogenated hydrocarbons such as chloroform, dichloromethane, ethylene dichloride, etc.; ethers such as diethyl ether, tetrahydrofuran, dioxane, etc.; amides such as dimethylformamide, etc.; dimethylsulfoxide; and water. It is possible to use a mixture comprising two or more solvents.

PM may be obtained by methyl esterifying L-phenylalanine in the presence of an acid catalyst, and in general, it is obtained as an addition salt with said acid, and therefore by neutralizing and subsequently extracting it with one or more of the above-mentioned solvents, it may be directly used in the reaction. The concentration of PM is not particularly restricted. Thus, for example, 0.1 to 0.8 M/litre is employed.

The N-formyl-L-aspartic acid anhydride may be used in the reaction as it is or may be dissolved or suspended in the aforesaid solvent after adding acetic acid or formic acid and formic acid, and then used in the reaction. The concentration thereof is not particularly restricted. Thus, for example, 0.2 to 4 M/litre is used.

The amount of the acetic acid, or the acetic acid and formic acid, used in the process of this invention is preferably as high as possible in view of the N-formyl-L-aspartic acid anhydride. However, if it is too high, the  $\alpha$ : $\beta$  ratio is lowered, and therefore 10 to 150% (by weight) based on the

reaction solvent is preferably employed. The formic acid, when used, is preferably used in an amount of not greater than 30% (by weight) based on the acetic acid and not greater than 5% (by weight) based on the reaction solvent.

The molar ratio of N-formyl-L-aspartic acid anhydride to PM in the process of this invention is not particularly restricted. Thus, for example, 0.5 to 5.0 is employed. The reaction temperature is preferably not higher than 100°C, more preferably not higher than 80°C, so as to prevent racemization of the product.

While there is no particular restriction on the reaction time, the reaction rate between the N-formyl-L-aspartic acid anhydride and the L-phenylalanine methyl ester is large and thus it is not necessary to conduct the reaction for a prolonged time. Generally, depending upon the reaction temperature, a retention time of within 6 hours is satisfactory. Furthermore, it is also possible to conduct the reaction in a plurality of vessels in several stages (cascade mode).

By this invention, since it is possible to greatly enhance the  $\alpha:\beta$  ratio and also greatly improve the yield of the desired end product,  $\alpha$ -APM and also since the reaction is conducted in a continuous mode, it is efficient also in respect of its apparatus and thus it is extremely advantageous from an industrial point of view.

The following Examples illustrate the invention. Reference will be made in the Examples to the single Figure of the drawing, showing a reactor having an inlet 1 for PM solution, and inlet 2 for N-formyl-L-aspartic acid anhydride, an overflow 3, and a stirrer driven by a motor 4.

#### EXAMPLE 1

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:toluene = 40:100) in a concentration of 0.5 M/litre, at a rate of 28.6 g/hr, and N-formyl-L-aspartic acid anhydride at a rate of 28.6 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 6.0:1.

#### EXAMPLE 2

To a reactor having an effective capacity of 800 ml, as shown in Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:formic acid:toluene = 25:5:1000) in a concentration of 0.5 M/litre at a rate of 400 ml/hr and N-formyl-L-aspartic acid anhydride at a rate of 28.6 g/hr, while the reaction mixture was completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 5.7:1.

#### EXAMPLE 3

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:toluene = 100:100) in a concentration of 0.5 M/litre at a rate of 400 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 28.6 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 15°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 7.0:1.

#### EXAMPLE 4

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:toluene = 20:100) in a concentration of 0.5 M/litre, at a rate of 800 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 57.2 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 5.7:1.

#### EXAMPLE 5

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:toluene = 40:100) in a concentration of 0.2 M/litre, at a rate of 400 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 11.4 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 5.7:1.

#### EXAMPLE 6

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and butyl acetate (acetic acid:butyl acetate = 40:100) in a concentration of 0.4 M/litre at a rate of 400 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 22.9 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 5.7:1.

#### COMPARATIVE EXAMPLE 1

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in toluene in a concentration of 0.5 M/litre at a rate of 400 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 28.6 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to over-

flow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 0.9:1.

#### COMPARATIVE EXAMPLE 2

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:toluene = 5:100) in a concentration of 0.5 M/litre at a rate of 400 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 28.6 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 3.7:1.

#### COMPARATIVE EXAMPLE 3

To a reactor having an effective capacity of 800 ml, as shown in the Figure, were continuously fed a solution of PM in acetic acid and toluene (acetic acid:toluene = 900:100) in a concentration of 0.5 M/litre at a rate of 400 ml/hr, and N-formyl-L-aspartic acid anhydride at a rate of 28.6 g/hr, while the reaction mixture was being completely mixed by stirring, and while the reaction mixture was continuously withdrawn by being allowed to overflow. During this time, the temperature of the reaction mixture was maintained at 30°C. The retention time of the PM and N-formyl-L-aspartic acid anhydride was 120 minutes.

The For- $\alpha$ -APM and For- $\beta$ -APM in the overflowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha:\beta$  ratio was found to be 4.3:1.

#### COMPARATIVE EXAMPLE 4(BATCH REACTION)

7.2 g of an N-formyl-L-aspartic acid anhydride was added to 100 ml of a solution of PM in acetic acid and toluene (acetic acid:toluene = 40:100) in a concentration of 0.5 M/litre, and the mixture was stirred at 30°C for 2 hours.

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The For- $\alpha$ -APM and For- $\beta$ -APM in the over-flowing reaction mixture were quantitatively determined by HPLC, and their  $\alpha$ : $\beta$  ratio was found to be 3.5:1.

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#### **Claims**

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1. A process for the production of N-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester, characterized in that N-formyl-L-aspartic acid anhydride is reacted with L-phenylalanine methyl ester in a reaction solvent and in the presence of acetic acid or acetic acid and formic acid and in a complete mixing type continuous reaction mode.

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2. A process according to claim 1 wherein the reaction solvent is toluene or ethyl acetate.

3. A process according to claim 1 or 2, wherein the amount of acetic acid or acetic acid and formic acid present is from 10 to 150% by weight based on the reaction solvent.

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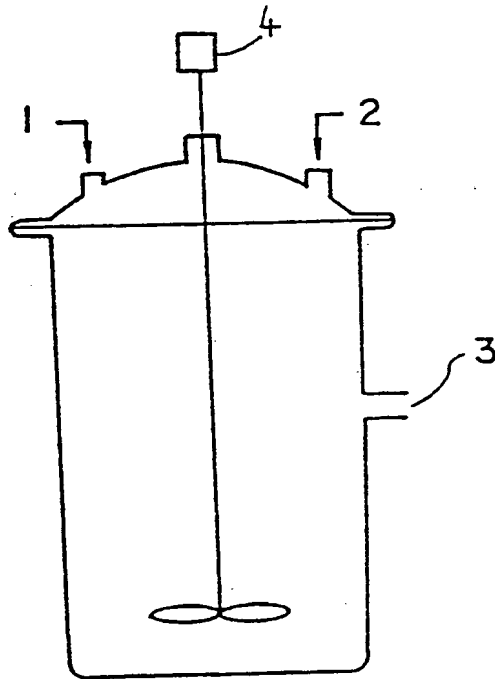
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## EUROPEAN SEARCH REPORT

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EP 86 30 9057

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	FR-A-2 249 872 (MONSANTO) * Whole document in particular page 3, lines 24-27 * -----	1-3	C 07 K 5/06 C 07 K 1/02
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			C 07 K 5/00 C 07 K 1/00
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 07-02-1989	Examiner MASTURZO P.
<b>CATEGORY OF CITED DOCUMENTS</b>			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			
T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			